

# Synthesis of sugar esters in solvent mixtures by lipases from *Thermomyces lanuginosus* and *Candida antarctica* B, and their antimicrobial properties

Manuel Ferrer<sup>a</sup>, Juan Soliveri<sup>b</sup>, Francisco J. Plou<sup>a,\*</sup>, Nieves López-Cortés<sup>a</sup>,  
Dolores Reyes-Duarte<sup>a</sup>, Morten Christensen<sup>c</sup>, José L. Copa-Patiño<sup>b</sup>, Antonio Ballesteros<sup>a</sup>

<sup>a</sup> Departamento de Biocatálisis, Instituto de Catálisis y Petroleoquímica, CSIC, Cantoblanco, 28049 Madrid, Spain

<sup>b</sup> Departamento de Microbiología y Parasitología, Universidad de Alcalá, Alcalá de Henares, 28871 Madrid, Spain

<sup>c</sup> Novozymes A/S, Novó Allé, 2880 Bagsvaerd, Denmark

Received 4 July 2003; accepted 11 February 2004

## Abstract

The lipases from *Thermomyces lanuginosus* (immobilized by granulation with silica) and *Candida antarctica* B (adsorbed on Lewatit, “Novozym 435”) were comparatively assayed for the synthesis of sugar esters by transesterification of sugars with fatty acid vinyl esters in 2-methyl-2-butanol:dimethylsulfoxide mixtures. We found that lipase from *C. antarctica* B is particularly useful for the preparation of 6,6′-di-acylsucrose, whereas *T. lanuginosus* lipase catalyzes selectively the synthesis of 6-*O*-acylsucrose. The granulated *T. lanuginosus* lipase retained more than 80% of its initial activity after 20 cycles of 6 h. Both lipases were similarly effective for the regioselective synthesis of 6′-*O*-palmitoylmaltose and 6-*O*-lauroylglucose. The effect of the synthesized sugar esters on the growth in liquid medium of various microorganisms (Gram-positive, Gram-negative and yeasts) was evaluated. 6-*O*-lauroylsucrose and 6′-*O*-lauroylmaltose inhibited the growth of *Bacillus* sp. at a concentration of 0.8 mg/ml, and of *Lactobacillus plantarum* at 4 mg/ml. Sucrose dilaurates and 6-*O*-lauroylglucose did not show antimicrobial activity, probably due to their low aqueous solubility. As regards the inhibition of yeasts, none of the tested carbohydrate esters inhibited significantly the growth of *Zygosaccharomyces rouxii* and *Pichia jadinii*.

© 2004 Elsevier Inc. All rights reserved.

**Keywords:** Carbohydrate esters; Sucrose esters; Maltose esters; Glucose esters; Lipases; Enzymatic transesterification; Enzyme granulation; Vinyl fatty acid esters; Antimicrobial activity

## 1. Introduction

Sugar fatty acid esters, synthesized from renewable resources such as fatty acids and carbohydrates, have broad applications in the food industry [1,2]. Other fields of application include cosmetics, detergents, oral-care products and medical supplies. Their antimicrobial [3], antitumoral [4] and insecticidal [5] properties have been reported and might open new markets. Sucrose esters and methyl glucoside esters, by far the most developed derivatives of this group, are being produced at about 4000 and 2000 Tm/year, respectively [6].

Regioselective acylation of carbohydrates is an arduous task due to their multifunctionality [7]. Sugar esters can be synthesized using either chemical or biological catalysts. Their current chemical synthesis is usually base-catalyzed at high temperatures, has a poor selectivity and gives rise to coloured side-products [8]. However, the enzyme-catalyzed processes are notably more selective [9]. For this purpose, lipases are the most useful enzymes. Furthermore, the two lipases most commonly used in sugar esters synthesis are those from *Thermomyces lanuginosus* (formerly *Humicola lanuginosa*) and *Candida antarctica* B.

We recently developed a new and simple process for the lipase-catalyzed acylation of sucrose [10] and other di- and trisaccharides [11]. The method was based on the use of mixtures of a tertiary alcohol (2-methyl-2-butanol) and a polar

\* Corresponding author. Tel.: +34 91 585 4869; fax: +34 91 585 4760.

E-mail address: [fplou@icp.csic.es](mailto:fplou@icp.csic.es) (F.J. Plou).

URL: <http://www.icp.csic.es/abg> (F.J. Plou).

solvent (dimethylsulfoxide) as reaction media, which represent a compromise between enzyme activity and sugar solubility. In this work, we have optimized the synthesis of various sugar mono- and diesters in solvent mixtures. We have compared the lipases from *T. lanuginosus* and *C. antarctica* B in terms of activity, regioselectivity and potentially reliable catalysts for sugar ester production.

An intensive effort is still being made in the screening of novel compounds able to inhibit or delay the growth of a range of microorganisms, for food and medical applications. In particular, sugar fatty acid esters display significant activity against several food and clinical isolates [3,12]. They have increasing interest due to advantages with regard to performance, consumers' health and environmental compatibility compared to petrol-derived standard products. By controlling the esterification degree, which may be modulated by the nature of the biocatalysts and the solvent composition, as well as the nature of fatty acid and sugar, it is possible to modify their properties. Taken this into consideration, the effect of the synthesized derivatives on the growth of a series of microorganisms involved in food spoilage was also investigated.

## 2. Experimental

### 2.1. Chemicals

Granulated lipase from *T. lanuginosus* and immobilized lipase from *C. antarctica* B (Novozym 435) were from Novozymes A/S. Maltose, glucose, molecular sieves (3 Å, 8–12 mesh) and 2-methyl-2-butanol (*tert*-amyl alcohol) were from Sigma. Sucrose and dimethyl sulfoxide (DMSO) were supplied by Merck. Vinyl laurate was from Fluka. Vinyl palmitate was from TCI (Tokyo, Japan). All other reagents and solvents were of the highest available purity and used as purchased.

### 2.2. Organisms

The microorganisms used in this study included *Bacillus* sp. CECT 40, *Pseudomonas fluorescens* CECT 378, *Staphylococcus aureus* CECT 240, *Erwinia carotovora* CECT 225T, *Escherichia coli* CECT 101, *Bacillus stearothermophilus* CECT 47, *Lactobacillus plantarum* CECT 220, *Zygosaccharomyces rouxii* CECT 1232 and *Pichia jadinii* CECT 1062. Microorganisms strains were maintained according the recommendations of the manual of the Spanish Type Culture Collection (CECT) (<http://www.uv.es/cect/>).

### 2.3. Enzymatic synthesis of sucrose esters

Sucrose laurate was synthesized by transesterification of sucrose (0.03 M) with vinyl laurate (0.3 M) in 2-methyl-2-butanol containing 20% of dimethylsulfoxide, in a 5 ml scale. Silica-granulated lipase from *T. lanuginosus* or the immo-

bilized lipase from *C. antarctica* B (Novozym 435) were used as biocatalysts (100 mg/ml). Reactions were performed at 40 °C with orbital shaking (100 rpm) in the presence of 3 Å molecular sieves (100 mg/ml). Reactions were followed by HPLC, using a SP-8810 pump (Spectra-Physics Inc.), a Nucleosil 100-C18 column (250 mm × 4.6 mm, Análisis Vínicos, Spain), maintained at 40 °C, and a refraction-index detector model 2410 (Waters). The mobile phase was a 90:10 (v/v) methanol:water mixture at 1.1 ml/min. The products were isolated by column chromatography, and fully characterized by spectroscopic techniques (NMR, IR, HR-MS) as previously described [10].

### 2.4. Enzymatic synthesis of maltose esters

Maltose esters were synthesized by transesterification of maltose (0.03 M) with vinyl laurate or palmitate (0.15 M) in 2-methyl-2-butanol containing 20% of dimethylsulfoxide, in a 5 ml scale. The reaction mixture contained 25 mg/ml of biocatalyst and 25 mg/ml of 3 Å molecular sieves. Reactions were performed at 40 °C with orbital shaking (100 rpm). Reactions were followed by HPLC, using a 9012 pump (Varian) and a Nucleosil 100-C18 column (250 mm × 4.6 mm, Análisis Vínicos, Spain), maintained at 40 °C. Detection was performed using an evaporative light-scattering detector DDL-31 (Eurosep) equilibrated at 55 °C. Methanol:water 95:5 (v/v) containing 0.1% acetic acid was used as mobile phase (flow rate 1.2 ml/min) for 7 min. Then, a gradient from this phase to pure methanol was performed in 1 min at the same flow rate. Methanol was held as mobile phase at 1.2 ml/min during 12 min. The products were purified by solvent precipitation and column chromatography, and fully characterized by spectroscopic techniques (NMR, IR, HRMS), as previously described [11].

### 2.5. Enzymatic synthesis of glucose esters

Glucose laurate was synthesized by transesterification of glucose (0.3 M) with vinyl laurate (0.3 M) in 2-methyl-2-butanol. Reactions were performed at 40 °C with orbital shaking (100 rpm) in the presence of biocatalyst (100 mg/ml) and 3 Å molecular sieves (100 mg/ml). Reactions were followed by HPLC under conditions described above for sucrose esters, but using a mobile phase 85:15 (v/v) methanol:water. The products were isolated as previously described [13]. Briefly, the mixture was filtered, washed with 2-methyl-2-butanol, evaporated under vacuum, and the solid (white powder) was recrystallized in acetone and dried in vacuum. The products were characterized by spectroscopic techniques (NMR, IR, HRMS).

### 2.6. Analysis of antimicrobial properties in liquid medium

The analysis of antimicrobial activity of the carbohydrate esters was performed using different liquid media accord-

ing of the microorganism tested: nutritive broth (meat extract 1 g/l, yeast extract 2 g/l, peptone 5 g/l, NaCl 5 g/l) for *Bacillus* sp., *P. fluorescens*, *S. aureus*, *E. carotovora*, *E. coli* and *B. stearothermophilus*; kimming broth (meat peptone 15 g/l, sodium chloride 1 g/l, glucose 19 g/l, glycerol 5 ml/l) for *L. plantarum*; and Saboureaud-glucose broth (tryptic digest of casein 5 g/l, meat peptone 5 g/l, glucose 20 g/l) for *P. jadinii* and *Z. rouxii*.

Stock solutions of carbohydrate fatty acid esters (80 mg/ml in methanol, except 6'-*O*-palmitoylmaltose that was dissolved in DMSO) were diluted to the desired concentration in the appropriate liquid media. The assays were prepared in test tubes adding 50  $\mu$ l of overnight culture microorganism suspension to the sugar esters solutions. Antimicrobial activity was tested after 24 h incubation at 28 °C (*Bacillus* sp., *P. fluorescens*, *E. carotovora*, *P. jadinii* and *Z. rouxii*), 37 °C (*S. aureus*, *E. coli* and *L. plantarum*) and 45 °C (*B. stearothermophilus*) by either visual appreciation of growth or measuring the turbidity at 600 nm. Percentage of antimicrobial activity in the problem test tube was calculated using as reference a test tube where no carbohydrate fatty acid ester was added, but containing either methanol or DMSO in the same proportion as in the problem test tube (100% growth).

### 3. Results and discussion

We synthesized a series of sugar esters, whose structure is summarized in Fig. 1, using an enzymatic method devel-

oped in our laboratory. The carbohydrate was pre-dissolved in a low amount of dimethylsulfoxide, and it was added to a tertiary alcohol (2-methyl-2-butanol). This increased substantially the solubility of the carbohydrate and avoided the inactivation of the biocatalyst [10]. Although this methodology has been assayed successfully with *T. lanuginosus* lipase immobilized in Celite [10,11] or granulated with silica [14], a comparative study in solvent mixtures with the widely used lipase from *C. antarctica* B adsorbed on Lewatit (Novozym 435) was performed in this work. The lipase from *T. lanuginosus* lipase was granulated with silica according to a method previously described [14] and is currently commercialized under the trade name Lipozyme® TL IM. Silica-granulation is a very simple and inexpensive immobilization methodology that gives rise to a high efficiency/cost ratio. Due to the easy handling of the granules, the resulting biocatalyst is easy to separate from the reaction mixture only by decanting [14].

#### 3.1. Synthesis of sucrose esters

The reaction medium contained 20% of dimethylsulfoxide in 2-methyl-2-butanol, which was previously demonstrated to be the optimal composition for the synthesis of sucrose monoesters using *T. lanuginosus* lipase [10]. Fig. 2 shows the HPLC chromatogram of the reaction mixture in the acylation of sucrose with vinyl laurate. As shown, the reaction with *T. lanuginosus* lipase is very selective towards the formation of monoester, namely 6-*O*-lauroylsucrose. This lipase

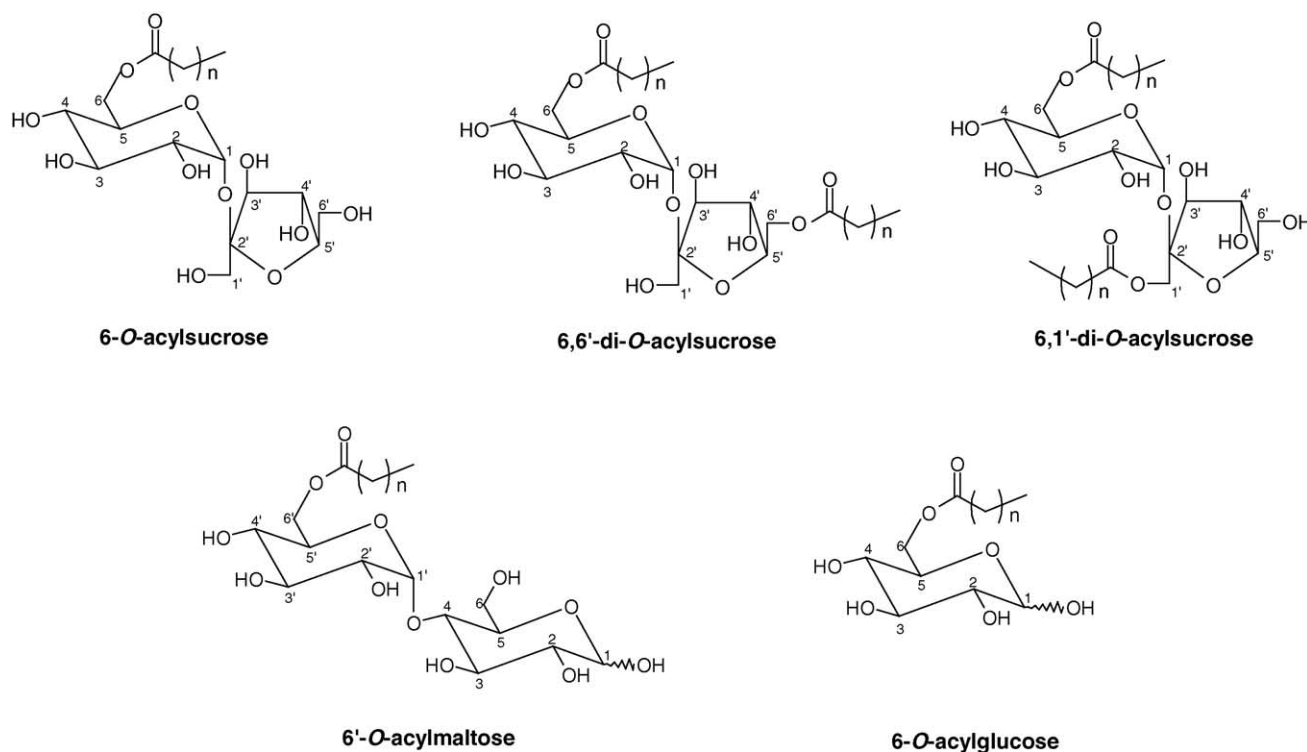


Fig. 1. Structures of the carbohydrate fatty acid esters studied;  $n = 10$  (in lauroyl), 14 (in palmitoyl).

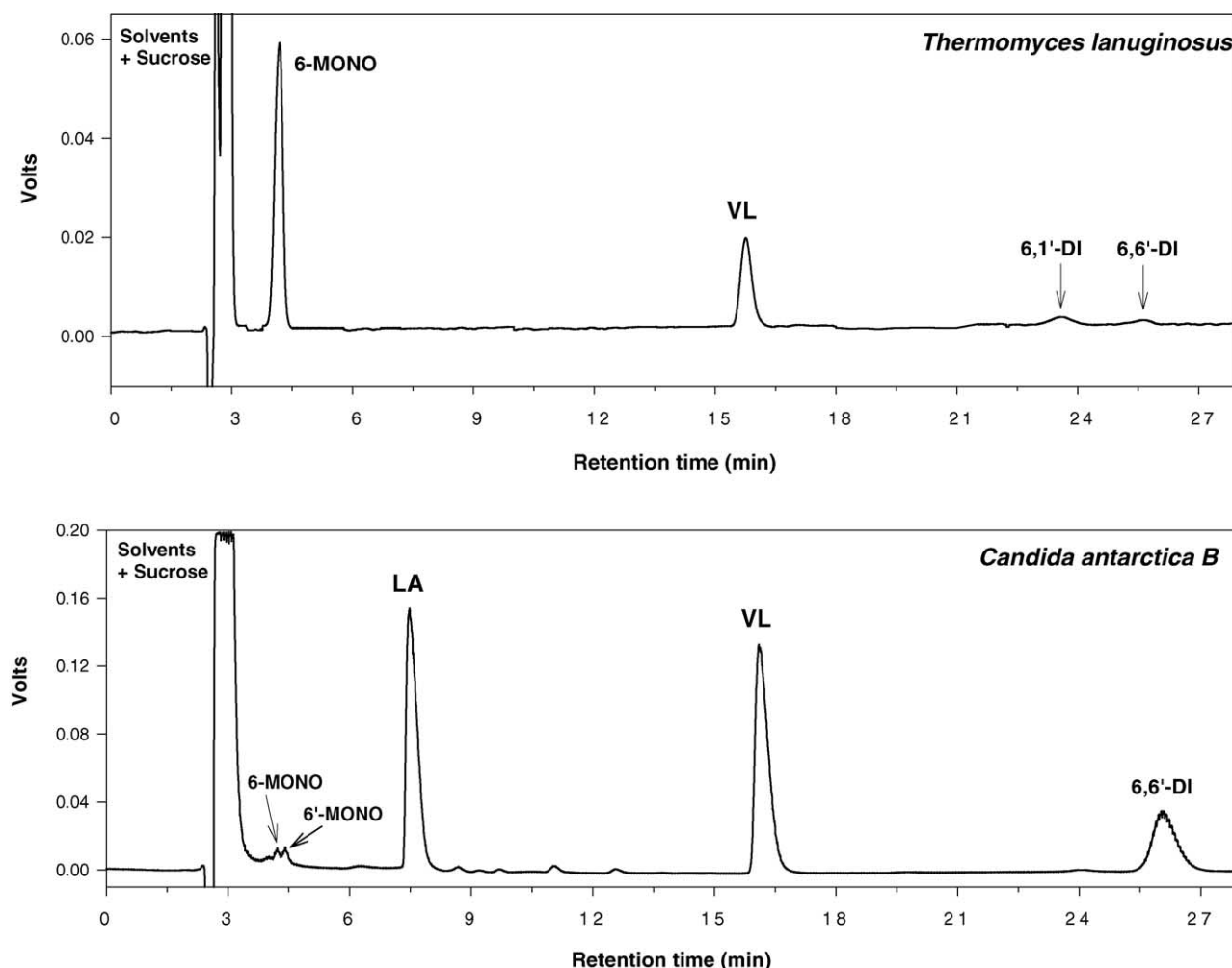


Fig. 2. Transesterification of sucrose with vinyl laurate in 2-methyl-2-butanol:DMSO 4:1 (v/v). HPLC chromatographic analysis of the reaction mixture using the lipases from *T. lanuginosus* and *C. antarctica B*. The peaks corresponding to monoesters (6-MONO- and 6'-MONO), lauric acid (LA), vinyl laurate (VL) and diesters (6,1'-DI and 6,6'-DI) are indicated. Conditions described in Section 2.

also gives a nearly equimolar mixture of the diesters 6,1'- and 6,6'-, whose yield may be increased lowering the percentage of DMSO [10]. In contrast, the formation of monoester with the lipase from *C. antarctica B* in 2-methyl-2-butanol/DMSO 4:1 (v/v) is only a minor process, compared with the synthesis of the diester 6,6'-di-*O*-lauroylsucrose. In addition, the monoester fraction is a mixture of the monoesters in the 6- and 6'-hydroxyl groups. In this context, Woudenberg et al. [15] reported that the reaction of sucrose with ethyl butanoate catalyzed by Novozym 435 in *tert*-butyl alcohol, gives rise to a mixture of 6- and 6'-monoesters in a molar ratio 1:1. In consequence, lipase from *T. lanuginosus* is convenient for the synthesis of the monoester 6-*O*-acylsucrose, whereas the lipase from *C. antarctica* can be employed for the selective synthesis of the 6,6'-diester.

We previously demonstrated with *T. lanuginosus* lipase that the DMSO percentage in the solvent mixture substantially modifies the final esterification degree [10]. Thus, at

DMSO concentrations  $\leq 10\%$  the formation of diesters is favoured, whereas at percentages higher than 15% the formation of diesters is minimized [10]. In this context, Bellot et al. [16] demonstrated in the esterification between glycerol and oleic acid that the solvent composition affects the selectivity, rate and substrate conversion at equilibrium. Using mixtures of *n*-hexane and 2-methyl-2-butanol of different composition, they observed that an increase in solvent polarity improves the selectivity towards the monoacylated products. The same effect was observed by us when increasing the percentage of DMSO.

In these processes, the formation of free fatty acid is a common feature as a consequence of the competence between the sugar and water for the acyl-enzyme intermediate. The amount of fatty acid depends strongly on the hydration degree of the different components used in the reactions, and especially on the water activity of the biocatalyst. Although Fig. 2 shows that the formation of fatty acid is negligible us-

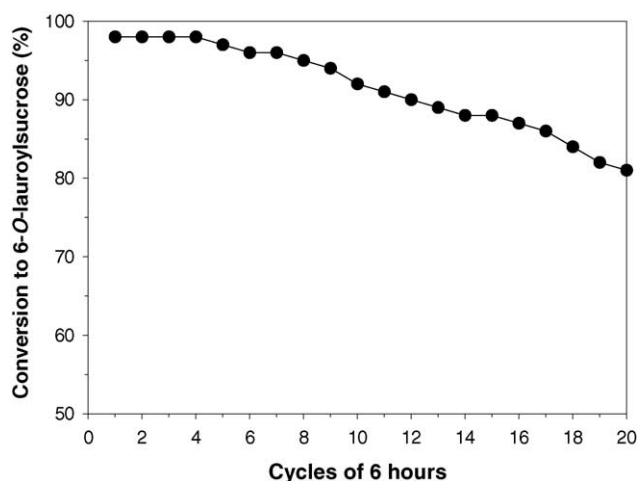


Fig. 3. Operational stability of silica-granulated *T. lanuginosus* lipase in the acylation of sucrose with vinyl laurate in 2-methyl-2-butanol:DMSO 4:1 (v/v) at 40 °C. One cycle reaction corresponds to 6 h. Reaction conditions for each cycle are described in Section 2. The  $a_w$  of the recycled biocatalysts was adjusted to 0.11 prior to each cycle.

ing *T. lanuginosus* lipase, we have observed the appearance of fatty acid in similar experiments with this biocatalyst.

To further characterize its potential applicability, the reuse of silica-granulated lipase from *T. lanuginosus* was studied in this process (Fig. 3). After a reaction cycle (6 h), the immobilized lipase was separated from the reaction mixture by filtration, washed with 2-methyl-2-butanol, dried under vacuum and equilibrated at 25 °C with the vapour phase of a saturated salt solution of lithium chloride ( $a_w = 0.11$ ). The conversion achieved in the first cycle was 98%. As shown, the conversion to sucrose monolaurate is above 80% after 20 cycles of 6 h.

### 3.2. Synthesis of maltose esters

Both lipases were also compared in the acylation of maltose with vinyl palmitate. In both cases, the primary hydroxyl at the C-6 of the non-reducing end glucose is selectively acylated [11,15]. Fig. 4 shows the time course of 6'-O-palmitoylmaltose synthesis with both lipases. The conversion is slightly higher with Novozym 435, although the reaction rate at the first stage of the process is a bit higher with *T. lanuginosus* lipase. Maltose is commercially available as monohydrate, and a high concentration of palmitic acid is obtained in both cases by lipase-catalyzed hydrolysis of the vinyl ester, even in the presence of molecular sieves. This is of practical importance, because a high excess of the acylating agent is required and the fatty acid formed needs to be eliminated from the final product.

### 3.3. Synthesis of glucose esters

The solubility of glucose (0.60 g/l at 30 °C, Ref. [13]) in 2-methyl-2-butanol is 3-fold higher than that of sucrose

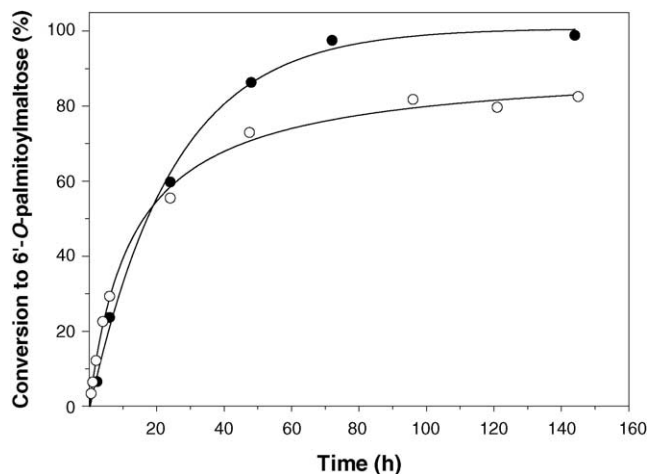


Fig. 4. Time-course of the acylation of maltose with vinyl palmitate in 2-methyl-2-butanol:DMSO 4:1 (v/v) catalyzed by: (●) lipase from *C. antarctica* B (Novozym 435); (○) silica-granulated lipase from *T. lanuginosus*. Conditions described in Section 2.

(0.21 g/l at 30 °C, Ref. [17]). This allowed us to perform the acylation of glucose in pure 2-methyl-2-butanol without the addition of DMSO as cosolvent. A slightly higher reaction rate was achieved with silica-granulated *T. lanuginosus* lipase (data not shown). The conversion was close to 90% in only 6 h. The compound obtained was 6-O-lauroylglucose [13]. The same selectivity was found with *C. antarctica* B lipase, in accordance with previous reports [18].

Fig. 5 shows comparatively the time course of glucose and sucrose acylation in pure 2-methyl-2-butanol catalyzed by silica-granulated *T. lanuginosus* lipase. The sucrose conversion is lower than 30% in 20 h, whereas a quantitative synthesis of 6-O-lauroylglucose is achieved.

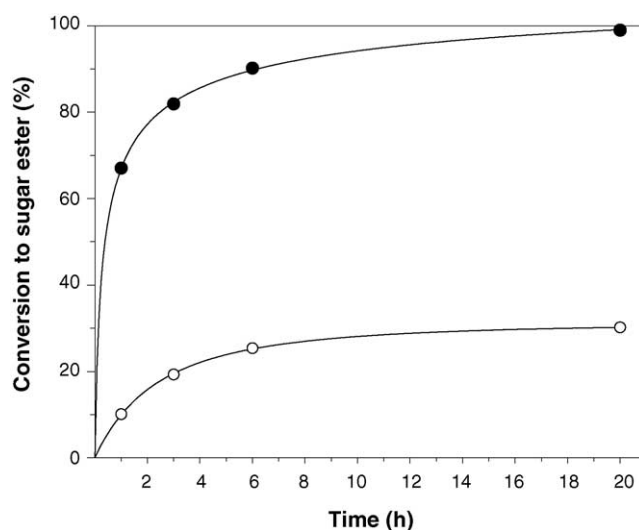


Fig. 5. Time-course of the acylation of glucose (●) and sucrose (○) with vinyl laurate in pure 2-methyl-2-butanol catalyzed by the silica-granulated lipase from *T. lanuginosus*. Conditions described in the Section 2.



Table 1

Screening of antimicrobial properties of a series of pure fatty acid esters of mono- and disaccharides<sup>a</sup>

| Compound                                       | <i>Bacillus</i> sp. | <i>Pseudomonas fluorescens</i> | <i>Staphylococcus aureus</i> | <i>Escherichia coli</i> | <i>Pichia jadinii</i> |
|--|---------------------|--------------------------------|------------------------------|-------------------------|-----------------------|
| 6- <i>O</i> -lauroylsucrose <sup>b</sup>       | — —                 | ++                             | ++                           | +/- —                   | ++/-                  |
| 6'- <i>O</i> -lauroylmaltose <sup>b</sup>      | — —                 | ++                             | ++                           | +/- —                   | ++/-                  |
| 6'- <i>O</i> -palmitoylmaltose <sup>c</sup>    | ++                  | ++                             | ++                           | ++                      | ++/-                  |
| 6,1'-di- <i>O</i> -lauroylsucrose <sup>d</sup> | ++                  | ++                             | ++                           | ++                      | ++                    |
| 6,6'-di- <i>O</i> -lauroylsucrose <sup>c</sup> | ++                  | ++                             | ++                           | ++                      | ++                    |
| 6- <i>O</i> -lauroylglucose <sup>c</sup>       | ++                  | ++                             | ++                           | ++                      | ++                    |

<sup>a</sup> (++) Positive growth; (++/-) growth more positive than negative; (+/- —) growth more negative than positive; (— —) no growth.<sup>b</sup> Tested at 2 mg/ml.<sup>c</sup> Tested at 0.25 mg/ml.<sup>d</sup> Tested at 1 mg/ml.

### 3.4. Antimicrobial properties of sugar esters

Sugar esters are widely used in Japan as antibacterial agents in canned drinks. Most of the previous studies on antimicrobial properties of sugar esters employed commercial derivatives [3,19,20]. These are constituted by complex mixtures of monoesters, diesters, triesters, etc. containing different regioisomers. In a few cases, the sucrose monoester fraction was separated from higher esters to assay antimicrobial activity, although no pure regioisomers were further isolated [21]. To our knowledge, this is one of the first studies of antimicrobial properties using pure carbohydrate fatty acid esters. In contrast with numerous works on properties of sucrose-based derivatives, few data is available characterizing other disaccharide esters, probably due to the lack of appropriate synthetic methods. We have recently investigated the effect of several di- and trisaccharides esters on the growth of *Streptococcus sobrinus*, a bacteria with a key role in the initiation of dental caries [22]. With regards to monosaccharides, Watanabe et al. [23] studied the effect of several glucose, fructose and galactose esters on the growth of *Streptococcus mutants*.

Conley and Kabara [24] demonstrated that the esterification of sucrose with fatty acids without antimicrobial activity gave rise to active derivatives against certain microorganisms. In general, Gram-positive were more susceptible than Gram-negative bacteria, whereas the inhibition of yeasts growth was lower than that of the other fungi [3]. Inhibition of Gram-positive bacteria by combination of sucrose esters with well-known antibiotics has been also reported [25].

We studied the effect of sugar head group (glucose, sucrose, maltose), length of the fatty acid (lauric, palmitic) and degree of substitution (monoester, diester) on antimicrobial properties. The compounds assayed are those represented in Fig. 1. In a first screening, we analyzed their effect on the growth in liquid medium of several microorganisms involved in food spoilage and poisoning (*P. fluorescens*, *Bacillus* sp.), and in a diversity of diseases (*S. aureus*, *E. coli*). *P. jadinii* was selected to represent higher eukaryotic microorganisms. Results are summarized in Table 1.

Sucrose diesters and glucose monoesters are notably less soluble in water than disaccharide monoesters, as a consequence of their higher hydrophobicity. For this reason, su-

crose diesters and glucose monoesters could not be evaluated at concentrations higher than 0.25 mg/ml, except for 6,1'-di-*O*-lauroylsucrose, that was assayed at 1 mg/ml. At these concentrations, their antimicrobial activity against all the microorganisms tested was negligible.

6-*O*-lauroylsucrose and 6'-*O*-lauroylmaltose inhibited the growth of *Bacillus* sp. and *E. coli*. This seems related with previous observations on the inhibition of growing *Bacillus subtilis* cells using commercial sucrose esters of various fatty acids [26]. Kato and Shibasaki [21] reported that fatty acid esters of sucrose inhibit the growth of *E. coli*. However, Hathcox and Beuchat [20] observed that the growth of *E. coli* was unaffected in tryptic soy broth containing 1 mg/ml of a commercial sucrose laurate.

We further studied in more detail the antimicrobial properties of the sucrose and maltose esters. A commercial sucrose laurate (L-1695) was also comparatively analyzed. This compound is basically a mixture of the 6- and 6'-monoesters, and contains additionally 21% of sucrose dilaurates. Other microorganisms involved in food spoilage (*B. stearothermophilus*, *L. plantarum*, *E. carotovora* and *Z. rouxii*), and consequently feasible causal agents of infections in humans, were also assayed.

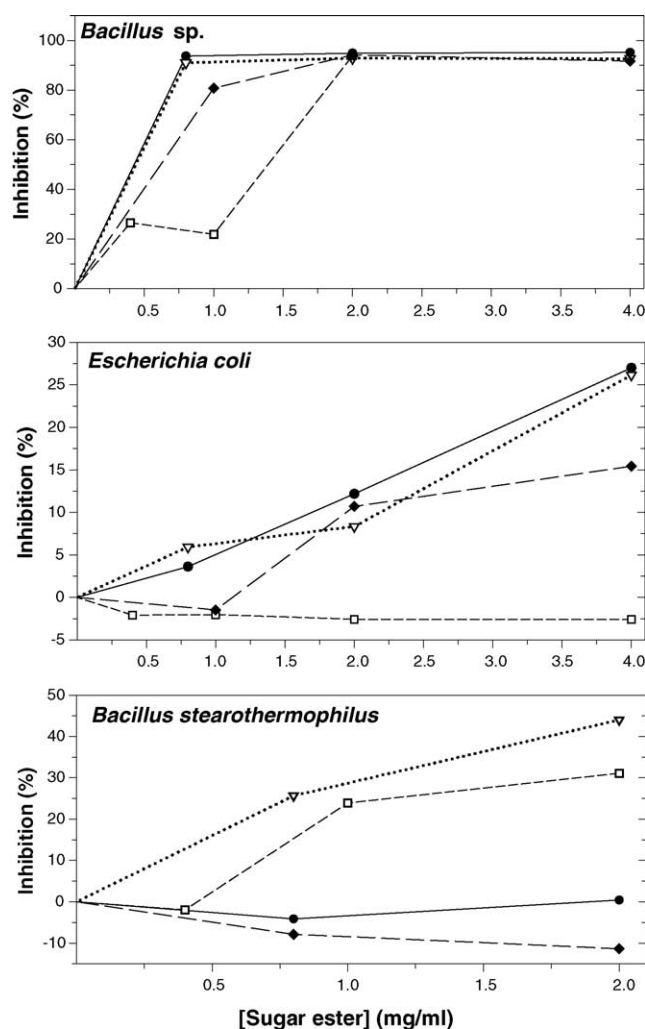
Our enzymatically-synthesized disaccharide esters showed interesting properties (Table 2). Among the bacteria tested, *Bacillus* sp. was the most susceptible. 6-*O*-lauroylsucrose and 6'-*O*-lauroylmaltose inhibited the growth of *Bacillus* sp., *E. coli* and *L. plantarum*. The two maltose esters showed a high bacteriostatic effect on *B. stearothermophilus*. Fig. 6 shows the effect of sugar ester concentration on inhibition of *Bacillus* sp., *E. coli* and *B. stearothermophilus*.

As regards the inhibition of yeasts, none of the tested carbohydrate esters inhibited the growth of *P. jadinii* and *Z. rouxii* at a significant extent. Although the concentrations showing antimicrobial activity in these experiments might be considered high (from 0.8 to 4 mg/ml), it is noteworthy that fatty acid esters of sucrose are commercially used as emulsifiers in foods at concentrations as high as 10 mg/ml (Ryoto® Sugar Ester Technical Information, Mitsubishi-Kagaku Foods Co., 1998). As a probe of their low toxicity, the maximum daily ingest of sucroesters is significantly high (20 mg/kg body weight).

Table 2

Screening of antimicrobial properties in liquid medium of a series of sugar fatty acid esters. The maximum percentage of inhibition and the corresponding ester concentration are shown

| Compound                       | Inhibition (%) | <i>Bacillus</i> sp. | <i>Pseudomonas fluorescens</i> | <i>Staphylococcus aureus</i> | <i>Erwinia carotovora</i> | <i>Escherichia coli</i> | <i>Bacillus stearothermophilus</i> | <i>Lactobacillus plantarum</i> | <i>Pichia jadinii</i> | <i>Zygosaccharomyces rouxii</i> |
|--------------------------------|----------------|---------------------|--------------------------------|------------------------------|---------------------------|-------------------------|------------------------------------|--------------------------------|-----------------------|---------------------------------|
| 6- <i>O</i> -lauroylsucrose    | 95 (4 mg/ml)   | —                   | 2.6 (4 mg/ml)                  | —                            | 22 (4 mg/ml)              | 26 (4 mg/ml)            | —                                  | 69 (4 mg/ml)                   | 5.0 (4 mg/ml)         | 2.5 (2 mg/ml)                   |
| 6'- <i>O</i> -lauroylmaltose   | 93 (0.8 mg/ml) | —                   | 1.9 (4 mg/ml)                  | —                            | 8.0 (4 mg/ml)             | 26 (4 mg/ml)            | 44 (2 mg/ml)                       | 68 (4 mg/ml)                   | 6.0 (4 mg/ml)         | 8.0 (2 mg/ml)                   |
| 6'- <i>O</i> -palmitoylmaltose | 94 (2 mg/ml)   | —                   | —                              | 2.3 (2 mg/ml)                | —                         | —                       | 31 (2 mg/ml)                       | 11 (2 mg/ml)                   | 1.6 (2 mg/ml)         | 3.5 (1 mg/ml)                   |
| L-1695                         | 94 (2 mg/ml)   | —                   | —                              | —                            | 27 (4 mg/ml)              | 15 (4 mg/ml)            | —                                  | 30 (4 mg/ml)                   | 7.0 (4 mg/ml)         | 2.0 (4 mg/ml)                   |

Fig. 6. Growth inhibition of *Bacillus* sp., *Escherichia coli* and *Bacillus stearothermophilus* by different concentrations of 6-*O*-lauroylsucrose (●, —), 6'-*O*-lauroylmaltose (▽, ···), 6'-*O*-palmitoylmaltose (□, ---) and sucrose laurate L-1695 (◆, - - -).

The mechanism of action of these carbohydrate esters is still unclear. Nevertheless, in *B. subtilis* cells growing actively, it has been observed that they cause changes in cellular morphology and induce autolysis processes that result in cell death [26,27]. It has been postulated that sugar esters reorganize the cellular membrane altering its permeability, which causes a loss of important metabolites [28].

#### 4. Conclusions

Lipase from *T. lanuginosus* is more convenient than *C. antarctica* B for the synthesis of sucrose 6-monoesters in solvent mixtures using vinyl fatty acid esters as acyl donors. Lipase from *C. antarctica* is especially interesting for the synthesis of the corresponding 6,6'-diesters. For the synthesis of maltose and glucose monoesters, both lipases are similarly useful. Disaccharide monoesters exhibit better performance

than monosaccharide monoesters, especially in terms of their higher solubility in aqueous medium and antimicrobial activity. Maltose esters could be used as alternative food emulsifiers because in addition to their surfactant properties [29], they exhibit antimicrobial properties comparable to sucrose esters.

## Acknowledgements

We thank Prof. Manuel Bernabé (Instituto de Química Orgánica, CSIC, Madrid, Spain) for help with NMR analysis. We thank Mr. Naoya Otomo (Mitsubishi-Kagaku Foods, Tokyo) for providing us a sample of sucroester L-1695. We are very grateful to Jordi Sucrana (SKW Biosystems, Barcelona, Spain) for technical help and fruitful suggestions. We thank CONACyT (Mexico) for a research fellowship to DRD. This work was supported by the European Union (Project BIO4-CT98-0363) and the Spanish CICYT (Project BIO2002-00337).

## References

- [1] Nakamura S. Using sucrose esters as food emulsifiers. *Oleochemicals* 1997;8:866–74.
- [2] Watanabe T. Sucrose fatty acid esters-past, present and future. *Foods Food Ingr J Jpn* 1999;180:18–25.
- [3] Marshall DL, Bullerman LB. Antimicrobial properties of sucrose fatty acid esters. In: Akoh CC, Swanson BG, editors. *Carbohydrate polyesters as fat substitutes*. New York: Marcel Dekker; 1994, 149–167.
- [4] Okabe S, Saganuma M, Tada Y, Ochiai Y, Sueoka E, Kohya H, et al. Disaccharide esters screened for inhibition of tumor necrosis factor- $\alpha$  release are new anticancer agents. *Jpn J Cancer Res* 1999;90:669–76.
- [5] Peterson JK, Harrison HF, Chortyk OT. Effects of various sucrose esters on weed seed germination and crop growth: structure-activity and dose-response relationship. *J Agric Food Chem* 1997;45:4833–7.
- [6] Hill K, Rhode O. Sugar-based surfactants for consumer products and technical applications. *Fett/Lipid* 1999;101:25–33.
- [7] Descotes G, Gagnaire J, Bouchu A, Thevenet S, Giry-Panaud N, Salanski P, et al. Preparations of esters, ethers and acetals from unprotected sucrose. *Polish J Chem* 1999;73:1069–77.
- [8] Polat T, Linhardt RJ. Synthesis and applications of sucrose-based esters. *J Surf Deterg* 2001;4:415–21.
- [9] Plou FJ, Cruces MA, Ferrer M, Fuentes G, Pastor E, Bernabé M, et al. Enzymatic acylation of di- and trisaccharides with fatty acids: choosing the appropriate enzyme, support and solvent. *J Biotechnol* 2002;96:55–66.
- [10] Ferrer M, Cruces MA, Bernabé M, Ballesteros A, Plou FJ. Lipase-catalyzed regioselective acylation of sucrose in two-solvent mixtures. *Biotechnol Bioeng* 1999;65:10–6.
- [11] Ferrer M, Cruces MA, Plou FJ, Bernabé M, Ballesteros A. A simple procedure for the regioselective synthesis of fatty acid esters of maltose, leucrose, maltotriose and n-dodecyl maltosides. *Tetrahedron* 2000;56:4053–61.
- [12] Bergsson G, Arnfinnsson J, Steingrímsson O, Thormar H. Killing of Gram-positive cocci by fatty acids and monoglycerides. *APMIS* 2001;109:670–8.
- [13] Tsavas P, Polydorou S, Fafila I, Voutsas EC, Tassios D, Flores MV, et al. Solubility of glucose in mixtures containing *t*-pentanol, dimethyl-sulfoxide, acids, esters and water. *J Chem Eng Data* 2002;47:807–10.
- [14] Ferrer M, Plou FJ, Pastor E, Fuentes G, Cruces MA, Andersen L, et al. Effect of the immobilisation method of lipase from *Thermomyces lanuginosus* on sucrose acylation. *Biocatal Biotransform* 2002;20:63–71.
- [15] Woudenberg M, Van Rantwijk F, Sheldon RA. Regioselective acylation of disaccharides in *tert*-butyl alcohol catalyzed by *C. antarctica* lipase. *Biotechnol Bioeng* 1996;49:328–33.
- [16] Bellot JC, Choisnard L, Castillo E, Marty A. Combining solvent engineering and thermodynamic modeling to enhance selectivity during monoglyceride synthesis by lipase-catalyzed esterification. *Enzyme Microb Technol* 2001;28:362–9.
- [17] Tsavas P, Polydorou S, Voutsas EC, Magoulas S, Naraghi K, Halling PJ. Sucrose solubility in mixtures of water, alcohol, ester and acid. *J Chem Eng Data* 2002;47:513–7.
- [18] Yan Y, Bornscheuer UT, Cao L, Schmid RD. Lipase-catalyzed solid-phase synthesis of sugar esters. Removal of byproducts by azeotropic distillation. *Enzyme Microb Technol* 1999;25:725–8.
- [19] Tsuchido T, Yokosula N, Takano M. Isolation and characteristics of a *Bacillus subtilis* mutant tolerant to the lytic action of sucrose esters of long-chain fatty-acids. *J Ferment Bioeng* 1993;75:191–5.
- [20] Hathcox AK, Beuchat LR. Inhibitory effects of sucrose fatty acid esters, alone and in combination with ethylenediamine tetraacetic acid and other organic acids, on viability of *Escherichia coli* O157:H7. *Food Microbiol* 1996;13:213–25.
- [21] Kato N, Shibasaki I. Comparison of antimicrobial activities of fatty acids and their esters. *J Ferment Technol* 1975;53:793–801.
- [22] Devulapalle KS, Gomez de Segura A, Ferrer M, Alcalde M, Mooser G, Plou FJ. Effect of carbohydrate fatty acid esters on *Streptococcus sobrinus* and glucosyltransferase activity. *Carbohydr Res* 2004;339:1029–34.
- [23] Watanabe T, Katayama S, Matsubara M, Honda Y, Kuwahara M. Antibacterial carbohydrate monoesters suppressing cell growth of *Streptococcus* mutants in the presence of sucrose. *Curr Microbiol* 2000;41:210–3.
- [24] Conley AJ, Kabara JJ. Antimicrobial action of esters of polyhydric alcohols. *Antimicrob Agents Chemother* 1973;4:501–6.
- [25] Thomas LV, Davies EA, Delves-Broughton J, Wimpenny JWT. Synergist effect of sucrose fatty acid esters on nisin inhibition of Gram-positive bacteria. *J Appl Microbiol* 1998;85:1013–22.
- [26] Tsuchido T, Svarachorn A, Soga H, Takano M. Lysis and aberrant morphology of *Bacillus subtilis* cells caused by surfactants and their relation to autolysin activity. *Antimicrob Agents Chemother* 1990;34:781–5.
- [27] Cho H-Y, Tsuchido T, Ono H, Takano M. Cell-death of *Bacillus subtilis* caused by surfactants at low concentrations results from induced cell autolysis. *J Ferment Bioeng* 1990;70:11–4.
- [28] Iwami Y, Schachtele CF, Yamada T. Effect of sucrose mono-laurate on acid production, levels of glycolytic-intermediates, and enzyme-activities of *Streptococcus mutans* NCTC-10449. *J Dent Res* 1995;74:1613–7.
- [29] Ferrer M, Comelles F, Plou FJ, Cruces MA, Fuentes G, Parra JL, et al. Comparative surface activities of di- and trisaccharide fatty acid esters. *Langmuir* 2002;18:667–73.